WORK PLAN AND METHODOLOGY

WORK PLAN

1. **Review of Literature.**

2. **Plant materials collection and authentification.**

3. **Pharmacognostical study.**
   - Microscopically study of root, root bark stem, stems bark, & leaves of two species.
   - Ash value of root, root bark stem, stems bark, & leaves of two species.
   - Extractive value of root, root bark stem, stems bark, & leaves of two species.

4. **Phytochemical study.**
   - Extraction of root, root bark, stem, stem bark, & leaves of two species by methanol.
   - Fluorescence analysis of the crude extracts.
   - Isolation of berberine from root, root bark stem, stem bark, & leaves of two species and determination of percentage yield.
   - Characterization of the isolated berberine.
   - Quantitative estimation of berberine in various crude extracts by HPTLC technique.

5. **Institutional Animal Ethical Committee. (IAEC) clearance.**

6. **Antibacterial study.**
   - Estimation of antibacterial activity.

7. **In vivo hepatoprotective study. (Pharmacological screening).**
   - Estimation of biochemical parameters such as ALAT, ASAT, ALP, LDH, TGL, cholesterol, total protein, albumin, Total bilirubin and direct bilirubin in serum shall be carried out.
   - Histopathological studies of liver shall be carried out.

METHODOLOGY

1. **Review of Literature.**
   
   Literature review is a continuous process from starting of the project to the final stage of the project. For literature review sources used are Journals which includes National, International, Patents, Internet, Books, Pharmacopeia etc.
2. **Plant materials collection and authentification.**

   The different parts (root, root bark, stem, stem bark and leaves) of both the plants shall be collected from Dotabata hill in Nilgiri district of Tamil Nadu. And shall be authenticated by comparing with the authentic specimen by botanist Dr. Baburaj Survey of Medicinal Plants, Central Council for Research in Homoeopathy Government Arts College Campus, Ootacamund.

3. **Pharmacognostical Studies.**

   Pharmacognostical studies shall be carried out according to the standard procedure and WHO guidelines.

4. **Photochemical Studies.**

   Phytotochemical studies shall be carried out according to the standard procedure and WHO guidelines.

5. **IAEC clearance.**

   Permission shall be taken from IAEC for the use of animals

6. **Antibacterial Study**

   In antibacterial studies the following shall be study:

   a) Both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) strain shall be used for the study.

   b) Nutrient agar media shall be used for the growth of bacteria for cylinder-plate method and nutrient broth media for serial dilution method.

   c) Methanolic extracts and the standard drug (Ampicillin trihydrate) shall be dissolved in dimethylsulphoxide (DMSO) at the concentration of 20 mg/ml and 1 mg/ml respectively.

   d) The results shall be calculated by inhibition of growth of bacteria.

7. **In vivo Hepatoprotective studies (Pharmacology screening).**

   a) *In vivo* hepatoprotective studies the evaluation of plants extracts using Country Made Liquor induced hepatotoxic model shall be carried out.